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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Steroid hormones are involved in the development and growth of breast cancer. Drugs, which inhibit estrogen action, are commonly used to inhibit breast cancer growth. Unfortunately, most advanced breast cancer becomes resistant to estrogen treatment. Recently, many steroid receptor coactivators have been discovered and found to potentiate the transcriptional activity of steroid receptors and enhance the expression of hormone response genes. In the SRC-1 family of coactivators, AIB1 is found amplified and/or over-expressed in breast cancer specimens. To evaluate the potential roles of the SRC-1 family of coactivators in mammary tumorigenesis *in vivo*, we proposed to generate transgenic mice over-expression of AIB1 (SRC-3) in mammary glands. To target the expression of AIB1 in mammary gland, we placed the AIB1 transgene under the control of the MMTV-LTR promoter. Two lines of transgenic mice expressing AIB1 have been generated. Studies on these transgenic mice will help understand the development and progression of breast cancer and provide a molecular basis for designing novel strategies to curb and, ultimately, cure breast cancer.

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INTRODUCTION

Steroid hormones play a central role in mammary gland development. The hormonal effects are mediated by estrogen and progesterone receptors, which act as hormone dependent transcription factors . Upon ligand binding, these receptors bind to the cognate hormone responsive elements on the target promoter and recruit co-activators and general transcription factors to regulate the expression of hormone responsive genes. Several coactivators which interact with estrogen and progesterone receptors have been cloned and extensively characterized in various laboratories, including ours. One of the co-activator, Steroid Receptor Co-activator (SRC-1), has been demonstrated by us to possess intrinsic histone acetyltransferase (HAT) activity. This property may facilitate localized chromatin remodeling and enhance assembly of basal transcription factors, resulting in the increase of transcription of hormone response genes. In addition to SRC-1, two other related genes of the same family have recently been identified and characterized. These are TIF-2(GRIP-1/SRC-2) and AIB-1(p/CIP/ACTR/RAC-3 /TRAM/SRC-3). Like SRC-1, these two coactivators have also been demonstrated to possess HAT activity and to interact with members of the nuclear receptor superfamily to enhance their abilities to transactivate the target genes.

Two salient points strongly implicate members of the SRC-1 family of coactivators as potential oncogenes for mammary tumorigenesis. Firstly, our published results indicate that ablation of SRC-1 gene by homologous recombination in mice severely compromised mammary gland development in response to estrogen and progesterone. Secondly, AIB-1/SRC-3 is amplified in 10% of breast cancer patients and is over-expressed in 64% of primary breast cancer samples. Since these family of coactivators are essential for the maximal expression of hormone response genes, we hypothesize that ectopic over-expression of these genes may play a role in the initiation, progression or transition of tumor cell growth from a hormone dependent to a hormone independent state. To test our hypothesis, we will generate transgenic mouse lines over-expressing either SRC-1 or AIB-1/SRC-3 and assess whether over-expression of these genes will result in mammary gland hyperplasia. We expect the results obtained from these studies will address whether over-expression of this family of co-activators play a key role in breast tumor formation.

SIGNIFICANCE

AIB-1 is a member of the SRC-1 family of transcriptional coactivators which have been shown to be essential for the maximal activation of hormone responsive genes. This family of transcription factors, not only interacts with members of the large nuclear receptor superfamily, but also possesses HAT activity and interacts with other coactivators including CBP/P300 and P/CAF. Therefore, over-expression of AIB-1 in breast cancer cells may not only perturb the expression of many estrogen, progesterone and other hormonal regulated genes, but may also affect the signal tranduction pathways which depend on CBP/P300 and P/CAF. Therefore, the deregulated expression of AIB-1 or SRC-1 may have a profound effect on the growth and development of mammary gland. To investigate how members of the AIB-1 family influence growth and development of mammary gland, we propose to use transgenic mice as a model system. Transgenic mouse lines over-expressing AIB-1 in the mammary gland will be generated. These lines will be used to assess whether over-expression of the AIB-1 will be sufficient to induce mammary tumorigenesis.

BODY

Expression of the AIB-1 in transgenic lines using MMTV-KCR-AIB-1 transgenic construct

Previously, we have obtained two transgenic founder lines, 2694 and 9845, expressing the AIB-1 in the mammary gland. However, the expression level of AIB-1 transgene is only 2 to 3 fold morethan that of the endogenous mP/CIP (human AIB-1 homologoue). To further analyze the expression of AIB-1 during mammary gland development, we chose the line 9845 for the subsequent experiments. RNA samples were isolated from transgenic mice in different developmental stages and the corresponding expression levels were detected by RNase protection assays using a AIB-1 specific riboprobe and normalized by the cyclophilin expression. The expression level of AIB-1 transgene in line 9845 was found to be stable throughout different stages of development, including virgin, pregnancy and lactation stages (Fig.1). The expression level of AIB-1 in mammary glands of transgenic mice remain to be much lower than that detected in breast cancer samples even in the lactation stage.

Assessment of the effect of over-expression of AIB-1 in mammary gland growth and development

Although the transgenic line does not express the AIB-1 at a high level as demonstrated in breast cancer patient samples, it is important to analyze for the physiological perturbation that may ascribe to the expression of AIB-1 in transgenic line 9845. Mammary gland whole-mount at different stages of development, 4-month old virgin and 16 days pregnant, were used for the assessment of the effect of over-expression of AIB-1 in mammary gland development. In the 4-month old virgin and 16 days pregnant heterologous (+/-) transgenic mice whole-mount sections, the degree of mammary gland developments were similar to the age and stage-matched wild-type mice. Only a slight increase in the number of lateral ductal branches was observed in transgenic mice. The insignificant difference may be due to the fact that the expression level of the AIB-1 transgene is not high enough to achieve a dramatic morphological change (Fig.2).
In an attempt to enhance the expression level of AIB-1 in the line 9845, a homologous line was generated from this founder line. Mammary gland whole mount sections from the homologous (+/+) and heterologous (+/-) 10-week old virgin transgenic mice were then prepared. Consistently, a slight but consistent increase in the number of lateral ductal branches was observed in both homologous and heterologous transgenic mice when compared to the age-matched wild type mice. Nevertheless, there is no significant difference between homologous and heterologous transgenic mice (Fig.3).

Expression of the AIB-1 in transgenic lines using the modified MMTV-AIB-1 expression vector

In an effort to achieve higher levels of expression of AIB-1 transgene in mammary gland, we chose another MMTV-SV40 expression vector that is widely used for targeting the expression of transgenes in mammary gland (Fig.4A). One out of five founder mice was found to express the transgene. The expression level of this line (line 5258) is even lower than that of the line 9845 (Fig.4B). In summary, at present, we have failed to generate a line overexpressing AIB-1 to a high level as demonstrated in the breast cancer patient sample.

Alternative approach to over-express AIB-1

It is possible that mammary epithelial cell might not tolerate high levels of AIB-l expression, so we cannot over-express the AIB-1 transgene in a high level using the traditional constitutive expression method. To overcome this problem, an inducible system developed in our laboratory will be used. This system employs a transactivator and a target. The transactivator GLp65 is a chimic protein containing a mutated human progesterone receptor ligand binding domain fused with a yeast GAL4 DNA binding domain and a partial p65 protein activation domain. To generate the transactivator line, MMTV-KCR-GLp65, a transactivator was subcloned into a modified version of MMTV-KCR vector, which can drive a mammary gland specific expression. The KCR fragment contained the partial exon II, intron II, exon III and a endogenous polyadenylation signal derived from rabbit β globin gene. For the generation of the target, the AIB-1 transgene was placed under the control of four yeast transcription factor GAL4 binding sites (refereed as 17X4) and a TATA box. Crossing of the transactivator line with the target line can obtain a bitransgenic mice. With the administration of RU486, the regulator can then bind to the 17X4 UAS recognition sequences upstream of the target oncogene and induce the expression of AIB-1 transgene. The expression of AIB-1 in bitransgenic mice can be turned on by RU486 at a specific window during development and the effects of this coactivator on mammary gland oncogenesis can be monitored (Fig.5).

Generation of the transactivator lines

Four transactivator lines were obtained. Northern blotting analysis indicated that the lines 5277 and 5826 have the high expression levels in mammary gland. To determine the tissue specificity of transgene expression in line 5277, total RNA isolated from a variety tissues were used for Northern analysis (Fig.6). As expected, the transgene was abundantly expressed in the mammary gland, but not in other tissues. This profile of expression is in agreement with that of MMTV-driven transgenes. Thus, this line was used for the subsequent studies.

Examination of the inducible system

To test the inducibility of the regulatory transgenic line, the MMTV-transactivator line was first crossed with a available Int-2 target line. The expression of the Int2 transgene in mammary gland can be induced only in presence of RU486 (Fig.7), suggesting that this MMTV-transactivator line is tightly regulated and will be useful for the future studies. The AIB-1 target line is now in preparation and will be crossed with this MMTV-transactivator line.

ONGOING EXPERIMENTS

Characterization of the inducible system using Int-2 as target

To elucidate the regulatory profile of the inducible system, the MMTV-transactivator line will be crossed with the Int-2 target line to generate the bitransgenic mice. Different dosages of RU486 and different induction times will be applied on the bitransgenic mice and the corresponding expression profiles of transgene and the morphological perturbations will be monitored.

Generation of AIB-1 target line

We are currently generating the AIB-1 target line. Once this line is available, the transactivator line will be crossed with the target line to get the bitransgenic mice. And AIB-1 expression in mammary gland can be induced with administration of the RU468.

KEY RESEARCH ACCOMPLISHMENTS

1. Generated the homologous MMTV-AIB-1 transgenic line.
2. Analyzed the mammary gland phenotypes of heterologous and homologous transgenic mice expressing AIB-1.
3. Generated the MMTV-transactivator and target transgenic constructs.
4. Established four transgenic mice expressing transactivator (GLp65) predominantly in mammary gland.
5. Examined the inducible system using the Int-2 target line.

REPORTABLE OUTCOMES

1. Analyzed the mammary gland phenotypes of heterologus and homologous transgnic mice constitutively expressing AIB-1.
2. Established an inducible system for over-expression of transgene in mammary gland.

CONCLUSION

The effects of the AIB-1 overexpression in the mammary gland growth and development were assessed by using mammary gland whole-mounts at different developmental stages. A mild increase in the number of lateral ductal was observed in both heterologous and homologous transgenic mice when compared with the age and stage-matched wild-type mice. The low expression of AIB-1 may account for the insignificant morphological changes. In an attempt to overexpress the AIB-1 to a high level as demonstrated in the cancer patient samples, we are currently generating the inducible system expressing the AIB-1 transgene under the control of RU486. Establishment of the inducible system will facilitate the analysis of the potential role of AIB-1 on the breast cancer formation and progression. It will also provide the molecular basis for design novel strategies to curb and ultimately cure breast cancer.

REFERENCES

None

Fig. 1. **Detection of the AIB-1 transgene in transgenic mammary glands.** A) The schematic representation of the MMTV-KCR-AIB-1 construct. The AIB-1 gene was placed under the control of MMTV promoter. KCR: rabbit b-globin fragment consisting of exon 2, intron 2 and exon 3 (E2, I2 and E3, respectively); BGHpA: polyadenylation signal frrom bovine growth hormone gene. B) Ribonuclease protection assay of total RNA isolated from 8 week-old mammary glands of founder and wild type mice (WT) showed that line 2649 and 9845 expressed the AIB-1 gene. C) Ribonuclease protection assay of total RNA isolated from mammary glands at different developmental stages. The expression of AIB-1 is stable throughout these developmental stages.

Fig. 2. **Wholemount analyses of 4-month-old virgin and 16-day-old pregnant heterologous (+/-) transgenic mice.** Comparison of the transgenic mammary glands with that of the wild-type (WT) revealed that transgenic mice displayed a slight but consistent increase in the number of lateral ductal branches.

Fig.3. **Wholemount analyses of 10-week-old virgin heterologous (+/-) and homologous (+/+) transgenic mice.** A slight but consistent increase in the number of lateral ductal branches was observed in both homologous and heterologous transgenic mice when compared to the age-matched wild type mice (WT).

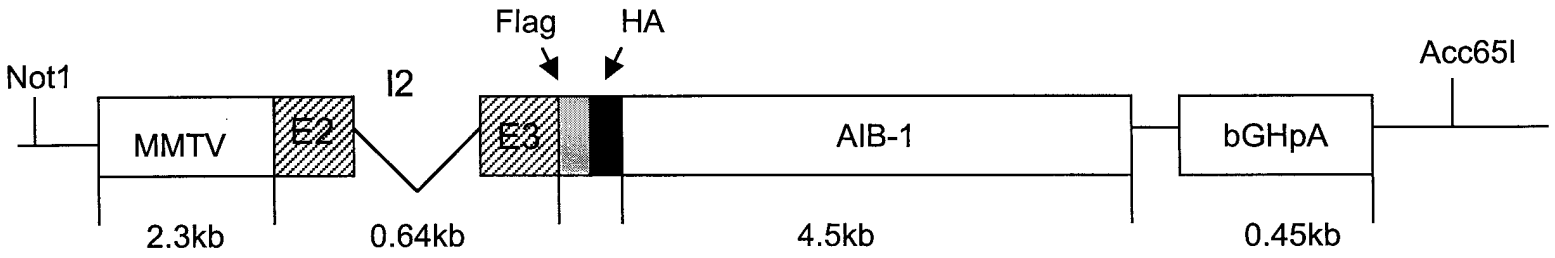
Fig. 4. **Detection of AIB-1 transgene in transgenic mammary glands.** A) The schematic representation of the MMTV-SV40-AIB-1 construct. The AIB-1 gene was placed under the control of MMTV promoter. The SV40pA polyadenylation signal allowed proper polyadenylation of the AIB-1 transcript generated. B) Ribonuclease protection assay of total RNA isolated from 8 week-old mammary glands of founder and wild type mice (WT) revealed that one out of the four founder line expressed the AIB-1 gene and the expression of line 5258 was lower than that of the line 9845.

Fig. 5. **Schematic representation of the MMTV-KCR-GLp65 and AIB-1 traget.** A) MMTV-KCR-GLp65: The transactivator GLp65 is a chimic protein containing a mutated human progesterone receptor ligand binding domain fused with a yeast GAL4 DNA binding domain and a partial p65 protein activation domain. It was placed under the control of MMTV-LTR promoter. B) AIB-1 traget: The AIB-1 gene was placed under the control of the rat collagenase TATA box and four Gal4 DNA binding sites (UAS).

Fig. 6. **Northern analyses to detect GLp65 transactivator.** Total RNA (10μg) isolated from tissues of virgin mice was hybridized with the transactivator probe. A) Four founders was found to express the GLp65 transactivator in mammary glands. B) For line 5277, the transactivator expressed predominantly in the mammary gland but not the other tissues.

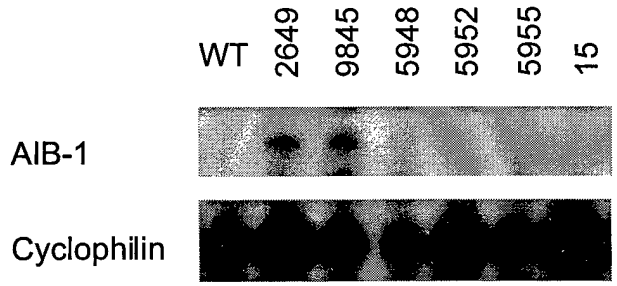
Fig. 7. **Detection of Int-2 target transgene in GLp65/Int-2 bigenic mice.** Ribonuclease protection assays of 10 μg of total RNA isolated from the bigenic and monogenic mammary glands revealed that the administration of RU485 but not Placebo to bigenic mammary glands was able to induce the expression of Int-2 transgene. Cyclophilin probe was used as internal standards to control for RNA loading. BG: bigenic (GLp65/Int2); MG: monogenic mice (Int2).

A. MMTV-KCR-AIB-1



B.

MMTV-KCR-AIB-1
Founder mice



C.

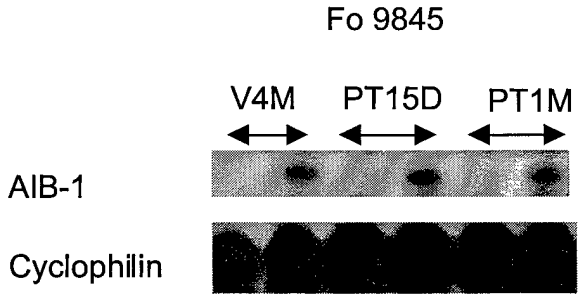


Fig.1

Mammary Gland Whole Mount from AIB-1 transgenic mice

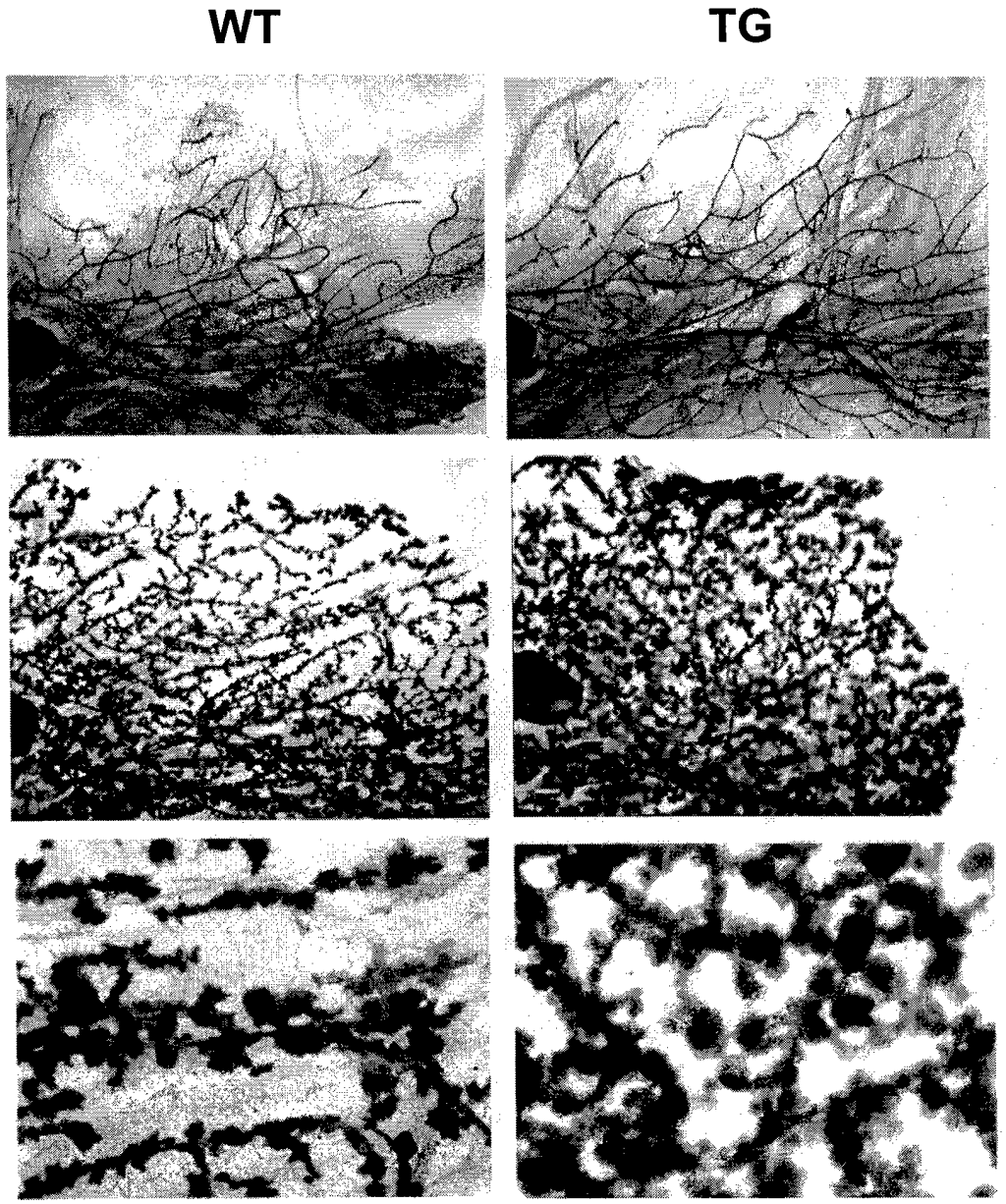


Fig.2

Mammary Gland Whole Mount from AIB-1 transgenic mice

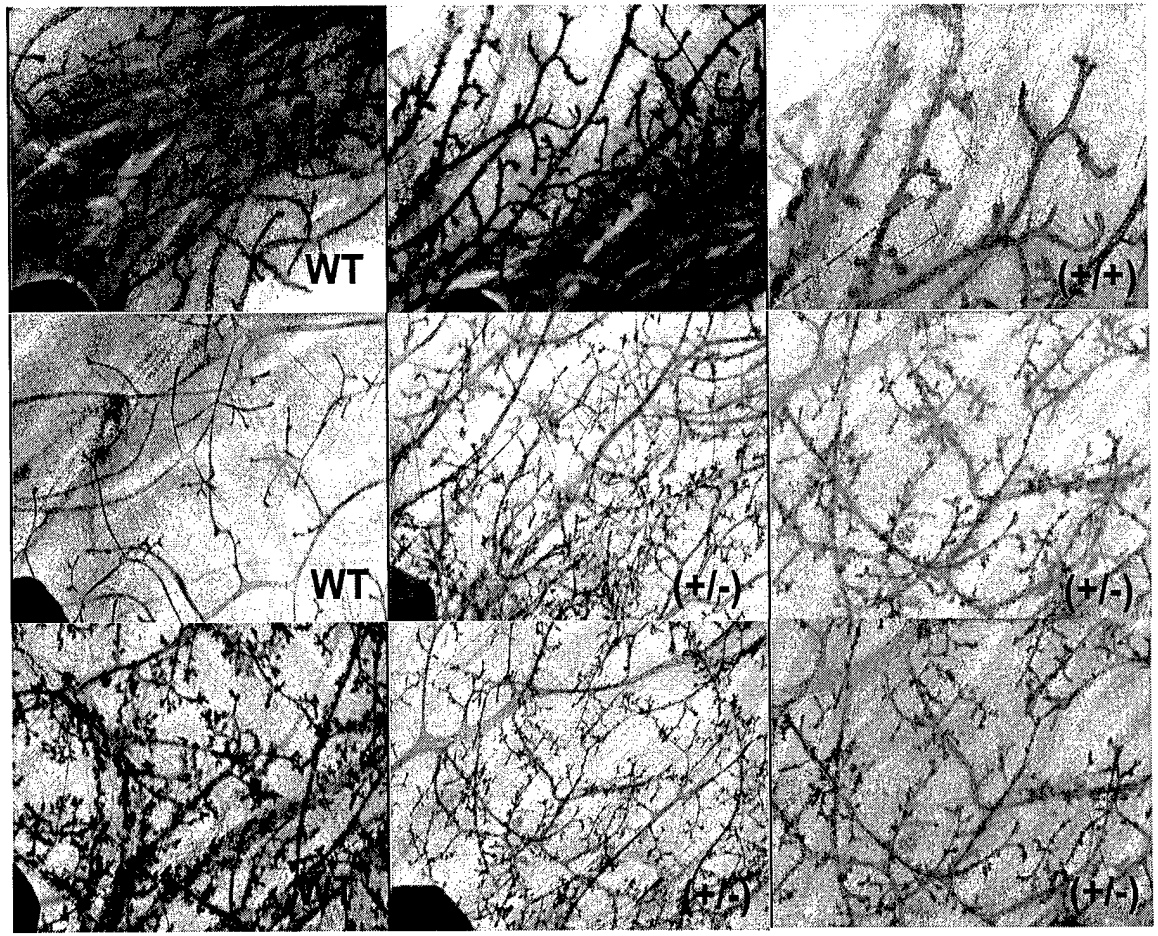
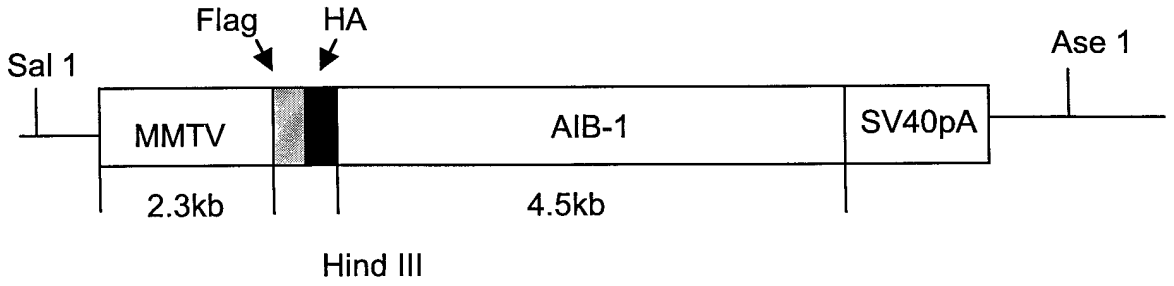


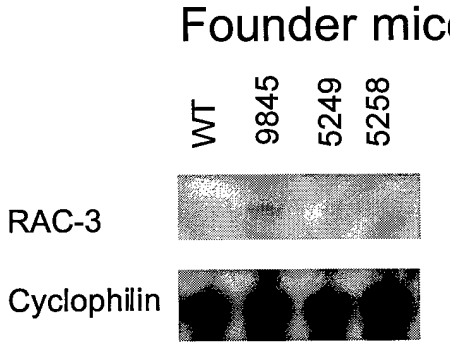
Fig.3

MMTV-SV40-AIB-1

A.



B.



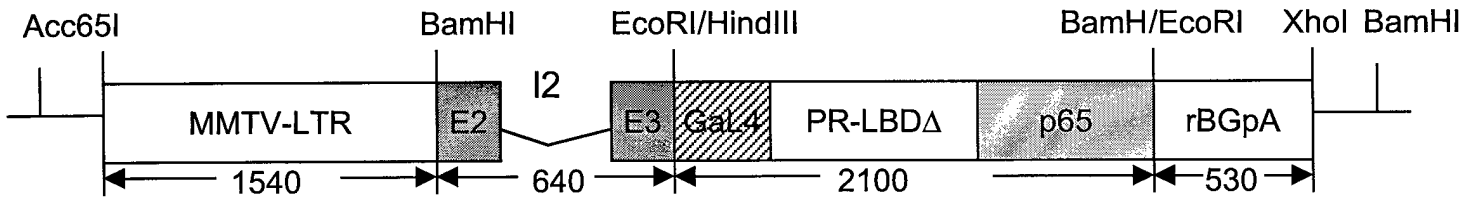
MMTV-SV40-AIB-1

F5248 (+/-) No expression
F5249 (+/-) No expression
M5252 (+/-) No expression
F5258 (+/-) Low expression
F5803 (+/-) No expression

Fig.4

A.

Transactivator MMTV-KCR-GLp65



B.

Target

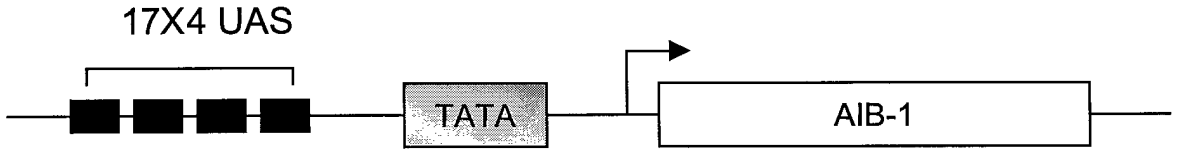


Fig.5

MMTV-KCR-GLp65 transgenic mice

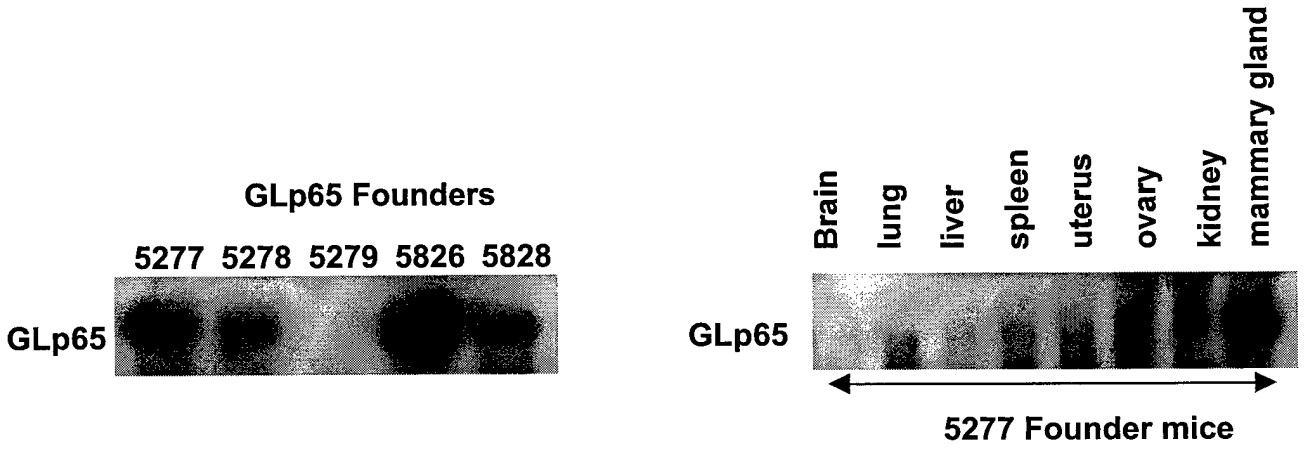
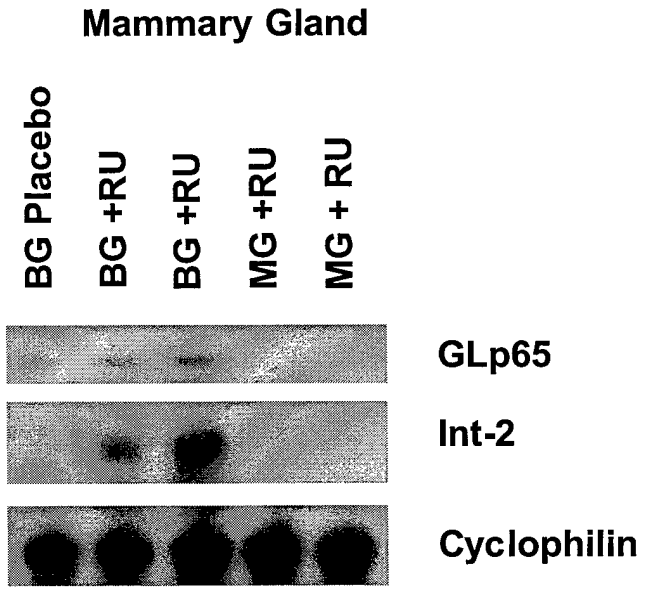


Fig.6

GLp65 (5277Founder) × Int-2 ♀

4 wks BG/MG virgin + RU486 or Placebo for 7 days



BG: (+/-, +/-)
MG: (-/-,+/-)

Fig.7